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PRINCIPAL INVESTIGATOR: Wen-Hwa Lee, Ph.D.

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13. ABSTRACT *(Maximum 200 words)*

The objective of the program is to train highly qualified doctoral students in the genetic, cellular, and molecular basis of Breast Cancer. The training in Breast Cancer research that students obtain will provide the momentum and scientific expertise for future discoveries in this important field. The training program, conducted within the Molecular Medicine Ph.D. Program, is administered by a select group of faculty whose research projects are intimately involved in breast cancer. An additional goal of the program is to promote synergistic interactions between the various laboratories engaged in breast cancer research. Breast cancer meetings, Molecular Medicine Distinguished Seminar Series are integral parts of the training program for students supported by the Breast Cancer Training Program. The major strengths of the program are the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty are organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth, differentiation and molecular genetics. During the reporting period, research by the students supported by the training program resulted in the publication of 32 peer-reviewed articles.

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PI - Signature Date

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INTRODUCTION

1. Brief Description of the Training Program and Its Objectives

The ongoing goal of the program is to establish at the University of Texas Health Science Center in San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. The most important goal of the program is to train highly qualified Ph.D. students in the genetic, cellular, and molecular basis of Breast Cancer. It is our expectation that the background in Breast Cancer Biology these students obtain will lead to significant future discoveries. To date, a total of 31 publications relevant to breast cancer has been achieved by the students supported by the training program.

The training program is conducted within the Molecular Medicine Ph.D. Program by a select group of faculty whose research projects are relevant to breast cancer. An additional goal of the program is to promote synergistic interactions between the various laboratories engaged in breast cancer research. An important event is the Annual Breast Cancer Symposium held in San Antonio. All students supported by the program were required to attend. Finally, an outstanding Molecular Medicine Seminar Series sponsored by the Department of Molecular Medicine was also a requirement for all trainees. The following seminars in this series were pertinent to breast cancer:

- Fall Semester,1997:

G. Steven Martin	"Transformation by Src and Ras"
Winship Herr	"Transcriptional regulatory mechanisms"
Glenn D Preswich	"New affinity probes for cell signalling"
Robert Benezra	"Mitotic checkpoint controls"
Richard Baer	"The functional properties of BRCA1"
Alan M. Weiner	"A viral model for chromosome fragility"
Michael Lieber	"Site-specific recombination"
Larry H. Thompson	"Recombination repair in mammalian cells."

- Spring Semester, 1998:

Joanna Groden	"Genomic stability and inherited predisposition to cancer"
Kenneth Kraqemer	"Recent studies on DNA repair and Xeroderma Pigmentosum"
Eric R. Fearon	"Colorectal cancer genetics and the DCC gene"
Carlo M. Croce	"Genetics of human cancer"
John D. Minna	"Molecular pathogenesis of lung cancer"
David B. Roth	"DNA cleavage and joining in V(D)J recombination"

- Fall Semster, 1998:

Xiaodong Wang	"Biochemical studies of apoptosis: putting a colorful puzzle together"
Richard Kolodner	"Multiple mechanisms of mutation suppression"
Jerry Shay	"The regulation of telomerase in aging and cancer"
Lorraine Symington	"Mechanisms of DNA double-strand break repair"
Nicholas K. Tonks	Signal transduction and protein tryosine dephosphorylation: from structure to function of protein tyrosine phophatases"
Charles M. Radding	"Molecular mechanisms of homologous recombination"
Joan Ruderman	"Cell cycle control"
Douglas Bishop	Assembly of meiotic recombination complexes"
Charles J. Sherr	"The ARF-p53 pathway in tumor surrveillance"
Dan Finley	"Targeting proteins for breakdown by the proteasome"
M. Andrew Hoyt	"Mechanisms and regulation of mitosis in S. cerevisiae"

Satya Prakash	"The DNA repair, protein degradation, and chromatin silencing activities of yeast Rad6 ubiquitin-conjugating enzyme"
James N. Ihle	"Signalling by the cytokine receptor superfamily"

One of the major strengths of the program is the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty are organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth, differentiation and molecular genetics. These faculty groupings are listed here, detailed descriptions of individual research programs were included in the original application.

A Breast Cancer Sub-Program

C. Kent Osborne, M.D.
John Chirgwin, Ph.D.
Suzanne Fuqua, Ph.D.
E. Lee, Ph.D.
W.-H. Lee, Ph.D.
Z. Dave Sharp, Ph.D.
Patrick Sung, Ph.D.

B. Growth Factor Sub-Program

Douglas Yee, M.D.
Gregory Mundy, M.D.
Robert J. Klebe, Ph.D.
Bettie Sue Masters, Ph.D.

C. Drug Development Sub-Program

Daniel Von Hoff, M.D.

D. Molecular Genetics Sub-Program

Robin Leach, Ph.D.
Peter O'Connell, Ph.D.
Alan E. Tomkinson, Ph.D.
Robert J. Christy, Ph.D.

Each of these faculty members maintains an active research program. The addition of Dr. Patrick Sung to the program faculty provides students with an opportunity to train in the laboratory of one of the world's experts in the biochemistry of DNA repair. His particular expertise in the reactions associated with recombinational repair of double-strand breaks in DNA. He is a recent recipient of a DOD Breast Cancer Research Idea Grant and Career Development Award both entitled "Interactions among BRCA1 and BRCA2 and components of the recomination machinery-I". He is an outstanding addition to the faculty of this training program. His research is directly applicable to breast cancer since perturbations of the

machinery that maintains genomic integrity play a major role in cancer initiation and progression.

In this progress report, the relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program is reviewed, and additional or updated information is provided regarding:

Research Support for Program Faculty
Listing of Supported Trainees
Project Summaries of upper level trainees
Appendix: Reprints of Trainee Publications

2. Relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program

The Breast Cancer Training Program was implemented within the context of the Molecular Medicine Graduate Ph.D. Program. The Molecular Medicine Ph.D. Program is a recently established interdisciplinary Ph.D. training program in the Graduate School of Biomedical Sciences at the UTHSCSA. For the academic year 1997-8, there is a total of 53 students enrolled in the Molecular Medicine Program -- 45 Ph.D. and 8 M.S. Of those 53 students, only six were supported by the Training Program in the Molecular Basis of Breast Cancer.

The Breast Cancer Training program takes advantage of the internationally recognized breast cancer research program existent in the institution for many years, and offers a unique opportunity for students interested in starting careers in breast cancer research. The participating scientists in this breast cancer program represent diverse departments including the Divisions of Medical Oncology, Hematology and Endocrinology in the Department of Medicine, and the Departments of Cellular and Structural Biology, Pathology and Biochemistry. In addition, the new University of Texas Institute of Biotechnology and the San Antonio Cancer Institute [SACI], an NIH-designated Cancer Center, represent outstanding resources for training opportunities in clinical and basic science research. The national and international reputation of the participating faculty serve to attract a large number of excellent applicants to the breast cancer research track in the Molecular Medicine program. The continuation of a Breast Cancer Specialized Program of Research Excellence (SPORE) grant to the institution documents the quality of breast cancer research available to trainees.

The rationale for administering the breast cancer training program in the Molecular Medicine Ph.D. program is based on several important criteria: [1] The Molecular Medicine curriculum is specifically designed to provide basic science training while integrating fundamental principles of molecular biology with modern medicine. A Molecular Medicine Core course provides students with the mechanisms underlying human disease and provides intensive review of specific diseases [including breast cancer] that may serve as models for how human diseases can be studied at the molecular genetic level. [2] The Molecular Medicine program requires the participation of both clinical and basic scientists in the training process. The inclusion of MDs on all student advisory committees insures that every graduate has a clear perspective on the clinical relevance of the basic research in their program, that in most instances, will serve as a guide for the project. [3] The Molecular Medicine program is an interdepartmental, interdisciplinary program that offers flexibility to students in terms of research laboratories, advisors and committee members. This arrangement offers a real potential for synergism in breast cancer research not possible in traditional department-

bound programs. In summary, our program offers a near perfect environment for Ph.D. training in breast cancer and has attracted many well-qualified applicants.

3. Research Support for Program Faculty

An essential component of maintaining a successful and aggressive training program in Breast Cancer Research is the continued research funding of the individual Program Faculty laboratories. Current funding for each member of the Program faculty is detailed in the table titled "Other Support". As can be readily seen from the table, the faculty have been extremely successful in obtaining research funding, including over $9,916,134 in direct costs for the 1997-1998 fiscal year. (See Research Support Table)

4. Listing of Supported Trainees

Trainees receiving support from the Training Program in the Molecular Basis of Breast Cancer Research are selected from among entering first year students in the Molecular Medicine Ph.D. Graduate Program. In subsequent years of their training, they may be maintained on the Training Program, or transferred to other funding sources, depending on the nature of their research interests, and the availability of grant support. The following trainees were supported on the Breast Cancer Training Program

Reporting Period 09/23/97 to 09/22/98

07/01/97 to O9/22/97

*John Leppard
Suh-Chin (Jackie) Lin
Shang Li
Hongyi Pan
*Sean Post
*Stephen Van Komen
#Lei Zheng
Qing Zhong
Frank Yuan, M.D.

* New to the program this year, see report below.
# A student previously supported by the program, see report below.

Record of Previous Year's Trainees:

Jim Fitzgerald	Graduated from the program with an M.S. degree.
Christa Hargraves	Left the program for academic reasons.
Zachary Mackey	Continues in the program as an upper level student [see report below].
Harold Pestana	Left the program for academic reasons.
Yuewei Qian	Graduated from the from the program with a Ph.D. Postdoc in James Maller's laboratory at the Howard Hughes Medical Institute at The University of Colorado School of Medicine. Dr. Qian's research involves understanding the cell cycle and cell proliferation. This is a problem that is relevant to all types of cancer, including those of the breast.

James Wang	Continues in the Molecular Medicine Ph.D. Program, currently funded by advisor's grant. Although no longer in the Training program, his work on the mechanism of viral latency is important in some cancers.
Linda deGraffenried	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Jennifer Gooch	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
David Levin	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Ernesto Salcedo	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below]. Ernesto was removed from the training grant since he elected to pursue work in a non-program facutly's laboratory [Dr. Steve Britt].
Jerry Alan Bates	Continues in the Molecular Medicine Program as Masters Student in the laboratory of Dr. Robert Clark, Professor and Chair of the Department of Medicine whose work in on signal transduction.
Jill Gilroy	Continues in the Molecular Medicine Ph.D. Program as Ph.D. Student in the laboratory of Dr. Hanna Abboud, Professor and Chief of the Nephrology Division in Department of Medicine. Ms. Gilroys work centers on signal transduction in kidney development.
Jonathen Mlocek	Resigned from the Molecular Medicine Ph.D. Program for personal reasons.
Ashby Morrison	Continues in the Molecular Medicine program as Ph.D. student in Dr. Kent Osborn's laboratory. She is working on identification and characterization of co-activators of the estrogen receptor and their role in the development of tamoxifen resistance during treatment of breast cancer.

The 1997-1998 academic year marks the fifth full year of operation for the Molecular Medicine Ph.D. Program, and the final one for the Training Program in the Molecular Basis of Breast Cancer Research. The availability of highly qualified applicants to the Molecular Medicine Program has proven to be excellent. Overall, about 100 applications were received for admission to the Fall 1998 entering class. Twelve students began classes in August of 1998. The total number of students at the start of the Fall semester 1998 in the Molecular Medicine Ph.D. Program at all levels was 53, which includes 18 women, and 4 minorities (1 black, 3 Hispanic students). Three minority students have been supported by the Training Program in the Molecular Basis of Breast Cancer Research.

5. Project Summaries of Ph.D. Trainees

Linda DeGraffenried ***Mentor -- Dr. Suzanne Fuqua***

Ms. deGraffenried's current project is to determine the cis-acting sequences responsible for the regulation of the human estrogen receptor gene. Deletion and site-directed mutagenesis of the ER promoter combined with transient transfection assays have revealed elements located proximal as well as distal to the primary transcriptional start site to be responsible. Mobility gel shift analysis suggests that a number of factors in whole cell extracts from ER-positive MCF-7 cells bind to the ER promoter between nucleotides -245 and -192, as indicated by the formation

of four specific protein/DNA complexes. This region of the promoter contains a GC box between -223 bp and -211 bp as well as a non-consensus binding site for Sp1 between -203 bp and -192 bp. Antibodies to the transcription factors Sp1 and Sp3 supershift two of the specific complexes. Cotransfection of expression plasmids for Sp1 and Sp3 with an ER promoter-driven luciferase reporter plasmid into Sp1-void *Drosophila* SL2 cells induces a one-hundred- and a thirty-fold activation of the ER promoter, respectively. Transient transfection assays using linker-scanner mutants of the ER promoter spanning -245 bp to -182 bp also suggest an important role for elements flanking the Sp binding sites in the regulation of ER gene transcription. A detailed elucidation of these elements as well as the DNA-binding proteins that mediate transcriptional response will be characterized.

This project is directly relevant to breast cancer. Elucidating the basis for regulation of ER expression is an important issue in breast cancer research.

deGraffenried, L.A. and Fuqua, S.A. 1998. An essential role for Sp1 and Sp3 in the regulation of estrogen receptor gene transcription. Journal of Biological Chemistry, in preparation.

Meeting Presentations and Abstracts

1. Hopp,T., **deGraffenried, L.A.,** and Fuqua, S.A.W. Variant forms of the estrogen receptor. Cambridge Symposia, 1997.

2. **deGraffenried, L.A.** and Fuqua, S.A. Enhancer regions involved in transcriptional regulation of the estrogen receptor gene in human breast cancer cells. 7th Annual Symposium on Cancer research in San Antonio, 1997.

3. **deGraffenried, L.A.** and Fuqua, S.A. Identification of regions involved in transcriptional regulation of the estrogen receptor gene in human breast cancer cells. 20th Annual San Antonio Breast Cancer Symposium, 1997.

4. **deGraffenried, L.A.** and Fuqua, S.A.W. Analysis of the role of the transcription factor Sp1 in transcriptional regulation of the estrogen receptor gene in human breast cancer. 8th Annual Symposium on Cancer Research in San Antonio, 1998.

5. **deGraffenried, L.A.** and Fuqua, S.A. The Sp1 transcription factor is involved in transcriptional regulation of the estrogen receptor gene in human breast cancer cells. 21st Annual San Antonio Breast Cancer Symposium, 1998.

David Levin ***Mentor -- Dr. Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. David is identifying the cellular functions involving the product of the LIG1 gene. Previous studies have implicated DNA ligase I in DNA replication and some pathways of DNA repair. During DNA replication, DNA ligase I presumably functions to join Okazaki fragments. However, under physiological salt conditions, DNA ligase I does not interact with DNA. It is Mr. Levin=B9s working hypothesis that DNA ligase I involvement in different DNA metabolic pathways is mediated by specific protein-protein interactions which serve to recruit DNA ligase I to the DNA substrate. To detect proteins that bind to DNA ligase I, David has fractionated a HeLa nuclear extract by DNA ligase I affinity chromatography. PCNA was specifically retained by the DNA ligase I matrix. To confirm that DNA ligase I and PCNA interact directly, Mr. Levin found that in vitro translated and purified recombinant PCNA bind to the DNA ligase I matrix. In similar experiments, he has shown that DNA ligase I interacts with a GST (glutathione S transferase)-PCNA fusion protein but not with GST. Using in vitro translated

deleted versions of DNA ligase I, Mr. Levin determined that the amino terminal 120 residues of this polypeptide are required for the interaction with PCNA. During DNA replication PCNA acts as a homotrimer that encircles DNA and tethers the DNA polymerase to its template. He showed that DNA ligase I forms a stable complex with PCNA that is topologically linked to a DNA duplex. Thus, it appears that PCNA can also tether DNA ligase I to its DNA substrate. A manuscript describing these studies has been published in the Proc. Natl. Acad. Sci. U.S.A.

In addition to interacting with PCNA, the amino terminal domain of DNA ligase I also mediates the localization of this enzyme to replication foci. To determine whether these are separable functions David fine mapped the region that interacts with PCNA and, in collaboration with Dr. Montecucco's group, the region required for recruitment to replication foci. Since the same 19 amino acids are necessary and sufficient for both functions and the same changes in amino acid sequence inactivate both functions, we conclude that DNA ligase I is recruited to replication foci by its interaction with PCNA. A manuscript describing these studies has been published in the EMBO
Journal.

In recent studies, Mr. Levin has a constrcted a mutant version of DNA ligase I that does not interact with PCNA. Importantly the amino acid substitutions do not affect the catalytic activity of DNA ligase I. By transfecting cDNAs encoding the mutant and wild type DNA ligase I into a DNA ligase I-mutant cell line, we will determine the biological significance of the DNA ligase I/PCNA interaction in DNA replication and DNA repair.

This project is relevant to breast cancer since problems with DNA replication and repair will undoubtedly be involved in the development of all tumors at some stage in their progression.

Publications;

Mackey, Z.B., W Ramos, DS Levin, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. Molec. Cell. Biol. 17, 989-998.

Tomkinson, A.E. and DS Levin Mammalian DNA ligases. Bioessays. 18, 803-901 (1997)

Levin, D.S. W Bai, N Yao. and M O'Donnell and AE Tomkinson. 1997 Interaction between DNA ligase I and Proliferating Cell Nuclear Antigen; implications for Okazaki fragment DNA metabolism. Proc. Natl. Acad. Sci. U.S.A. 94, 12863-12868.

Montecucco, A., R Rossi, DS Levin, R Gary, MS Park, TA Motycka, G Ciarrocchi, A Villa, G Biamonti and AE Tomkinson. 1998 DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen: Identification of a common targeting mechanism for the assembly of replication factories. EMBO J. 17, 3786-3795

Shang Li ***Mentor -- Dr. Wen-Hwa Lee***

Mutations of the *BRCA1* gene predisposes women to the development of breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein whose cellular function is poorly understood. The C-terminal region of the BRCA1 protein contains an activation domain and two repeats termed BRCT (for BRCA1 C-terminal). In his recent work, Mr. Li identified a BRCT-interacting protein previously identified as CtIP, a protein that interacts with the C-terminal-binding protein (CtBP) of E1A. Together, CtIP and CtBP are postulated to form a transcription corepressor complex. The ability of BRCA1 to transactivate the p21 promoter can be

inactivated by mutation of the C-terminal conserved BRCT domains. To explore the mechanisms of this BRCA1 function, the BRCT domains were used as bait in a yeast two-hybrid screen. A known protein, CtIP, a co-repressor with CtBP, was found. CtIP interacts specifically with the BRCT domains of BRCA1, both *in vitro* and *in vivo*, and tumor-derived mutations abolished these interactions. The association of BRCA1 with CtIP was also abrogated in cells treated with DNA-damaging agents including UV, γ-irradiation and adriamycin, a response correlated with BRCA1 phosphorylation. The transactivation of the p21 promoter by BRCA1 was diminished by expression of exogenous CtIP and CtBP. These results suggest that the binding of the BRCT domains of BRCA1 to CtIP/CtBP is critical in mediating transcriptional regulation of p21 in response to DNA damage.

This project is directly relevant to breast cancer since it involves the study of a protein whose function appears to central to the mobilizing the response of cells to DNA damage. Perturbations in the systems that maintain genomic integrity underlie initiation and progression of most cancers, including those of the breast.

Publications:

Chen C-F, **S. Li, Y. Chen**, P-L Chen, ZD Sharp, and W-H Lee. 1996 The Nuclear Localization Sequences of the *BRCA1* Protein Interact with the Importin-a Subunit of the Nuclear Transport Signal Receptor. **J. Biol. Chem.**, 271: 32863-32868 *Note: The three authors in bold contributed equally to this work.*

Liu, CY, A Flesken-Nikitin, **S. Li,** YY Zeng, and W-H. Lee. 1996. Inactivation of the mouse *Brca1* gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. Genes Dev. **10**:1835-1843.

Li, S, C-Y Ku, A. Farmer, Y-S Cong, C-F Chen, and W-H Lee. Identification of a novel cytoplasmic protein that specifically binds to nuclear localization signal motifs. J. Biol. Chem. 273:6138-6189 (1998).

Li, S, P-L Chen, T Subramanian, G Chinnadurai, G. Tomlinson, CK. Osborne, ZD Sharp, and W-H Lee Dissociation of BRCA1 Binding to CtIP upon DNA Damage Mediates p21 Expression. Submitted to J. Biol. Chem.

Zachary Mackey ***Mentor -- Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. In this project we are intending to identify the cellular functions involving the product of the LIG3 gene. Mammalian cell lines with reduced DNA ligase III activity exhibit spontaneous genetic instability and increased sensitivity to DNA damaging agents. We have cloned human and mouse cDNAs encoding DNA ligase III. In both mouse and humans, we have identified two forms of DNA ligase III cDNA that differ at their 3' end and encode polypeptides with different C-termini. At the site where the cDNA sequences diverge, the nucleotide sequence resembles consensus splice donor/acceptor sequences. We have confirmed that these cDNAs represent alternatively spliced products from the same gene by cloning and analysis of the 3' end of the mouse LIG3 gene. Analysis of DNA ligase III expression by northern blotting demonstrated that this gene is highly expressed in the testes. Using RT-PCR, we have examined the expression of the two forms of DNA ligase III cDNA in mouse tissues and cells. One form of DNA ligase III mRNA, DNA ligase III-a is ubiquitously expressed. In contrast, expression of DNA ligase III-b mRNA is restricted to the testis. During spermatogensis, DNA ligase III-b mRNA expression occurs during the latter stages of meiotic prophase. This restricted expression pattern suggests that DNA ligase III-b mRNA may have a

specific role in the completion of meiotic recombination. In support of this idea we have shown that DNA ligase III-a interacts with the DNA strand break repair protein Xrcc1 whereas DNA ligase III-b does not. We suggest that the DNA ligase III-a/Xrcc1 complex functions in DNA repair in both somatic and germ cells whereas DNA ligase III-b functions in meiotic recombination. A manuscript describing these studies has been published in Molecular and Cellular Biology.

A unique feature of the DNA ligases encoded by the LIG3 gene is an amino terminal zinc finger that binds to DNA single-strand breaks. This motif is not required for DNA joining vitro or for the functional complementation of an E. coli DNA =ligase mutant. However, the presence of this motif allows DNA ligase III to interact with and join nicked DNA molecules at physiological salt concentrations. Using site-directed mutagenesis, we have identified amino acid residues within the catalytic C-terminal domain that are required for interaction with nicked DNA. Our current working model is that the DNA ligase III zinc finger functions in vivo to displace another enzyme, poly (ADP-ribose) polymerase (PARP) from the nicks. A manuscript describing these studies has been submitted to the Journal of Biological Chemistry.

This project is relevant to breast cancer since genomic instability is likely to be involved at many of the several stages of breast cancer progression leading to malignancy. Methods to intervene and stabilize the genome could prevent progression and spread of the disease. In addition, information about DNA repair processes in normal and cancer cells may lead to the development of treatment regimes that more effectively kill cancer cells and minimize damage to normal tissues and cells.

Publications:

Wang, Y.-C.J., WA Burkhart, ZB Mackey, MB Moyer, W Ramos, I Husain, J Chen, JM Besterman and AE Tomkinson. 1994 Mammalian DNA ligase II is highly homologous with Vaccinia DNA ligase. Journal of Biological Chemistry 269, 31923-31928.

Husain, I., AE Tomkinson, WA Burkhart, MB Moyer, W Ramos, ZB Mackey, JM Besterman and J Chen. 1995 Purification and characterization of DNA ligase III from bovine testes. Journal of Biological Chemistry 270, 9683-9690.

Chen, J., AE Tomkinson, W Ramos, ZB Mackey, S Danehower, RA Schultz, JM Besterman and I Husain. 1995 Mammalian DNA ligase III: Molecular cloning, chromosomal localization and involvement in meiotic recombination during spermatogenesis. Molec. Cell. Biol. 15, 5412-5422.

Mackey, Z.B., W Ramos, DS Levin, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. Molec. Cell. Biol. 17, 989-998.

Tomkinson, AE and ZB Mackey. 1998 Structure and Function of Mammalian DNA ligases. Mutation Research. 407, 1-9.

Mackey, Z.B., Leppard, J., Menissier-deMurcia, Niedergang, C., Au, K., Chen, J. de Murcia, G. and Tomkinson, A.E. DNA ligase III is recruited to DNA strand breaks by a zinc finger motif homologous to that of poly (ADP-ribose) polymerase: Identification of two functionally distinct DNA binding regions within DNA ligase III (submitted, 1998)

Hongyi Pan ***Mentor -- Dr. Wen-Hwa Lee***

Mutations in the breast cancer susceptibility gene, *BRCA1*, is involved in the development of hereditary breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein whose function is not well understood. One of Mr. Pan's project is to identify BRCA1-interacting proteins. One protein identified in this screen, named AP12, is a zinc-finger-containing protein. Since AP12 has the hallmarks of a Krab-domain repressor protein, Mr. Pan first identified the recognition sequence necessary for DNA-binding. Next, he inserted this sequence into mammalian reporter constructs and demonstrated that AP-12 can, indeed, repress transcription. He is currently, performing experiments to determine if BRCA1 can influence AT-12-mediated repression. The hypothesis under test is that BRCA1 can influence positively or negatively the expression of a repertoire of AP12-regulated genes. This control function may be important in BRCA1-mediated suppression of breast cancer. In addition, Mr. Pan is working on a new protein that potentially links BRCA1 with Mre11, a protein critical to the repair of double-strand break in DNA. These results are in line with other data indicating a role for BRCA1 in modulating the DNA repair machinery during cellular responses to genotoxic insults.

This project is relevant to breast cancer since BRCA1 function is hypothesized to be involved in suppressing the formation of breast cancer.

Qing Zhong ***Mentor -- Dr. Wen-Hwa Lee***

One of Mr. Zhong's project in Dr. Lee's laboratory is a study of the tumor suppressor protein, TSG101. *tsg101* was identified as a tumor susceptibility gene by homozygous function inactivation of allelic loci in mouse 3T3 fibroblasts. To confirm its relevance to breast cancer that was originally reported, antibodies specific for the putative gene product were prepared and used to identify cellular 46 kDa TSG101 protein. A full size 46 kDa TSG101 protein was detected in a panel of 10 breast cancer cell lines and 2 normal breast epithelial cell lines with the same antibodies. A full-length *TSG101* mRNA was also detected using rtPCR. These results indicate that homozygous intragenic deletion of *TSG101* is rare in breast cancer cells. In more recent work, Mr. Zhong demonstrated that TSG101 is a cytoplasmic protein that translocates to the nucleus during S phase of the cell cycle. Interestingly, TSG101 is distributed mainly around the chromosomes during M phase. Microinjection of antibodies selective for TSG101 during G1 or S results in cell cycle arrest and overexpression leads to cell death. These data indicate that neoplastic transformation due to lack of TSG101 could be due to a bypass of cell cycle checkpoints.

This work is important for breast cancer research since it indicated that TSG101 may not be an important component for suppressing tumorigenesis, at least directly, since it is present in all the breast cancer lines. However, its overall role in tumorigenesis is significant.

Publications:

Zhong, Q, CF Chen, Y Chen, PL Chen, and WH Lee 1997 Identification of Cellular TSG101 protein in multiple human breast cancer cell lines. Cancer Res. 57, 4225-4228.

Zhong, Q, Y Chen, D Jones, W-H Lee 1998 Perturbation of TSG101 protein affects cell cycle progression. Cancer Res. 58; 2699-2702.

Ashby Morrison ***Mentor -- Dr. Kent Osborne***

Ms. Morrison worked in three labs, breast cancer research being the primary area of research in each lab. My first lab rotation, which was in the lab of Peter O'Connell, Ph.D., She was involved

in the preliminary work of locating a gene that when mutated may be involved in process of metastasis. The second lab rotation was done in the lab of Jolene Windle, Ph.D. During the months I spent in this lab I was exposed to the technique of using mouse models to study breast cancer. Specifically, my project involved transgenic and knockout mice to research the effects of oncogenes and tumor suppressors on breast cancer development. During her third lab rotation, in the lab of Kent Osborne, MD., Ms. Morrison was involved in a more clinical area of breast cancer research. Her project was to study the effects of varying levels of estrogen receptor coactivators and corepressors during tamoxifen treatment. Ms. Morrison was accepted into Dr. Osborne's laboratory where she continues to make good progress on the identification of estrogen receptor-associated proteins that are hypothesized to be co-activator/repressor proteins.

Jennifer Gooch ***Mentor -- Dr. Doug Yee***

Dr. Yee's laboratory is interested in the growth regulation of breast cancer cells by insulin-like growth factors (IGFs). Data from several laboratories had suggested that interleukin-4 (IL-4) and IGFs share common signaling pathways. Since it was known that IL-4 could directly inhibit breast cancer cell proliferation, Jennifer began examining the potential overlap of growth stimulatory and growth inhibitory signaling pathways in breast cancer cells.

Ms. Gooch first confirmed that IL-4 was inhibitory for breast cancer cells. This inhibition was dependent on expression of the IL-4 receptor and blocking antibodies to the receptor neutralized the effects of IL-4. She discovered that IL-4's growth inhibitory effects were dependent on cell proliferation. Quiescent cells were not affected by IL-4. Moreover, IL-4 induced apoptosis in estradiol-stimulated cells. She documented apoptosis by morphologic change, TUNEL assay, PARP cleavage, DNA laddering and generation of a sub-G1 peak by flow cytometry. Thus, she has shown that IL-4 inhibits breast cancer cell growth by inducing apoptosis to some, but not all, growth stimuli.

Because IL-4 and IGF-I share a common signaling pathway through insulin receptor substrate protein-1 (IRS-1), it is possible that this molecule coordinates both growth promoting and cell death signals. It is also possible that additional signals generated by IL-4 are responsible for its growth inhibitory effects. To date, she has documented Stat-6 activation by IL-4. She has shown that IL-4 treatment induces Stat-6 binding to a synthetic oligonucleotide in gel mobility shift assays. She has also shown that IRS-1 is activated by IL-4 in responsive cell lines. However, IL-4 differs from IGF-I in its kinetics of IRS-1 activation. While IGF-I rapidly phosphorylates IRS-1 to high levels followed by rapid dephosphorylation, IL-4 causes tonic levels of IRS-1 to appear in the cell. Furthermore, it appears that IRS-1 is rapidly degraded after IGF-I treatment, while such degradation does not occur after IL-4. Preliminary evidence suggests that IRS-1 may be ubiquitinated after IGF-I treatment, but not IL-4. Her future projects involve the detailed characterization of these pathways and determination of their contribution to IL-4's growth inhibitory effects.

Finally, she has shown that interferon-gamma (IFNg) stimulates Jak/Stat activation in human breast cancer cells. As in other epithelial tumors, activation of Stat-1 and Stat-3 appear to be growth inhibitory compared to their function in lymphocytes.

This project is relevant to breast cancer since intracellular signaling pathways are almost certainly involved in the growth stimulation at some stage of mammary cell tumor development or progression. Since growth inhibitory (IL-4) and growth stimulatory (IGF-I) pathways may be coordinated through a single molecule, the precise definition of the mechanism of IL-4 action, as compared to IGF-I action, could define molecular targets to inhibit breast cancer cell growth.

Publications:

Lee AV, Jackson JG, **Gooch JG**, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Synergistic enhancement of insulin-like growth factor signaling in human breast cancer: Estrogen regulation of insulin receptor substrate-1 *in vitro* and *in vivo.* In revision Mol. Endocrinology.

Gooch JL, Lee AV, Yee D. 1998 Interleukin-4 induces growth inhibition and apoptosis in human breast cancer cells. Can. Res. 58:4199-4205

Yee D, **Gooch JL**, Jackson JG. IGF-I, insulin, and IL4 activate IRS1 in human breast cancer cells: Differential IRS1 tyrosine phosphorylation by IGF-I is associated with increased MAPK and P13K activation. Proc AACR 38: A2910, 1997.

Jackson JG, **Gooch J**, Yenushe L, White MF, Lee AV, Yee D. Expression and activation of insulin receptor substrate-1 and -2 (IRS-1 and -2) in human breast cancer cells. 78th Annual Meeting of the Endocrine Society, 1996.

Gooch JL, Yee D, Lee AV. Ligand dependent degradation of insulin receptor substrate-1 in human breast cancer. Submitted to 1998 Endocrine Society Annual Meeting.

Lee AV, Jackson JG, **Gooch JL**, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Enhancement of the insulin-like growth factor signaling pathway by estrogen in human breast cancer. Submitted to 1998 Endocrine Society Annual Meeting.

Gooch JL, Jackson JG, Yee D. Interluekiin-4 induced apoptosis is associated with STAT6 activation, IRS-1 phosphorylation, and activation of the SAPK pathway in human breast cancer cells. Accepted at the 1998 Keystone Symposium on Signal Transduction.

Gooch JL, Van Den Berg, CL, Yee, D. 1998 Insulin-like growth factor 1 rescues breast cancer cell from chemotherapy-induced cell death: proliferative and apoptotic effects. Submitted to Breast Cancer Research and Treatment.

Jill Gilroy ***Mentor – Dr. Hanna Aboud***

Signal transduction pathways are a vital part of development, proliferation, and tumorigenesis. In my work, I am interested in the involvement of growth factors, primarily Platelet Derived Growth Factor (PDGF) and its receptor (PDGFR), in signaling pathways. PDGFRs are tyrosine kinase receptors and upon stimulation dimerize and autophosphorylate, which in turn induces many downstream signaling molecules including, Mitogen Activated Protien Kinase (MAPK), and Phosphatidylinositol 3-kinase (PI3K). One of my goals was to determine the role of PI3K and MAPK in mediating biological processes such as cell migration and proliferation by PDGFR activation. Activation of PI3K was assayed using thin layer chromtography of anti-phosphotyrosine immunoprecipitates. MAPK activation was measured by immune complex assay of MAPK immunoprecipitates and SDS-PAGE using anti-phospho-MAPK antibodies. Functional assays, chemotaxis and ^{3}H-thymidine assays, were also preformed to test for cell migration and proliferation respectively. Inhibitors of MAPK and PI3K were also used in these studies to further show the involvement of these pathways in the aforementioned biological processes.

This project is relevant to breast cancer since signal transduction pathways are a vital part of tumorigenesis.

Frank Yuan, M.D. ***Mentor -- Dr. Eva Lee***

The response of mammalian cells to DNA damage is complex, involving cell cycle arrest, DNA repair and, under certain conditions, apoptosis. Cells from individuals with the recessive disorder ataxia telangiectasia (AT) are hypersensitive to ionizing radiation. ATM (mutated in AT) protein contains a PI-3 kinase domain and is predominantly localized in the nucleus. c-Abl, a non-receptor tyrosine kinase, interacts with ATM and is a substrate of ATM kinase. Dr. Yuan demonstrated that ATM, c-Abl, and Rad51, a homologue of bacterial RecA protein required for DNA recombination and repair, can be co-immunoprecipitated from cell extracts. c-Abl interacts with and phosphorylates Rad51 *in vitro*. This phosphorylation enhances complex formation between Rad51 and Rad52, which functions with Rad51 in recombination and repair. After g-irradiation, an increase in both tyrosine phosphorylation of Rad51 and association between Rad51 and Rad52 occurs in wild-type cells but not in ATM-/- or c-Abl-/- cells. These findings implicate the ATM/c-Abl signaling pathway in promoting the assembly of the recombinational repair machinary.

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease characterized by microcephaly, immunodeficiency, chromosomal instability and high cancer risk. There are many common features shared by AT and NBS, including loss of cell cycle checkpoint and sensitivity to IR. It has been shown recently that the gene product of NBS, Nibrin, is a 95 kDa protein. We demonstrated that nibrin forms a stable complex with repair proteins Rad50 and Mre11. The Rad50/Mre11/nibrin complexes possess nuclease activities which are likely to be important for recombination, repair,and genomic stability.

Whether there is a biochemical link between ATM and p95 is being studied. This information will provide a biochemical basis for the A-T and NBS cellular phenotypes as well as the mechanism of IR sensitivity in these cells.

These projects are highly relevant to breast cancer since recent studies indicate that the protein product of breast cancer susceptibility gene BRCA1 interacts with Rad50 (Dr. Wen-Hwa Lee, personal communication). Furthermore, it has been reported that ATM carriers may have a higher risk of breast cancer.

Recombinase Rad51 plays a key role in homologous recombination. Multiple Rad51-interacting proteins including Rad52, Rad54 and RPA are also required for homologous recombination. Several labs have reported that the protein product of breast cancer susceptibility gene, BRCA2, interacts with Rad51 directly through its BRC domains. In normal cells, a redistribution of Rad51 protein, manifested as formation of Rad51 nuclear foci, is seen upon ionizing radiation (IR). We show that in cells harboring BRCA2 mutation, there is little IR-induced Rad51 foci formation. In addition, introduction of GFP-BRC/BRCA2 fusion protein but not GFP compromised IR-induced Rad51 foci formation. This study suggests a specific dependence of IR-induced nuclear distribution on BRCA2.

Trujillo, KM., **Yuan, S-S F.,** Lee, E. Y-H P., and Sung, P. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre 11, and p95. J. Biol. Chem. 273: 21447-21450.

Yuan, S.-S. F., Cox, L.A., Dasika, G. K. and Lee, E.Y.-H. P. Cloning and functional studies of a novel gene aberrantly expressed in RB-deficient embryos. (in revision for Dev. Biol.).

Yuan, S.-S. F., Chen, G., etc. Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl (submitted).

Yuan, S.-S. F., Lee, S.-Y., Chen, G., Song, M., Tomlinson, G.E., and Lee, E.Y.-H. P. BRCA2 is required for assembly of Rad51 complex in vivo. (submitted)

Suh-Chin(Jackie) Lin ***Mentor -- Dr. Eva Lee***

The tumor suppressor gene, p53, is frequently mutated in human tumors, including breast carcinoma. P53 null mice develop multiple spontaneous tumors, predominantly lymphoma and sarcoma, within the first 6 months of age. To establish a mouse model of p53-mediated mammary tumor development, a bigenetic approach employing the cre-loxp system was initiated by Ms. Lin. Through gene-targeting in embryonic stem (ES) cells, mice carrying floxed p53 genes in which exons 5 and 6 are flanked by the loxp sequence were generated. A second mouse line carrying a cre transgene under the control of mouse mammary tumor virus LTR (MMTV-cre) has also been generated. Floxed p53 mice were mated with MMTV-cre transgenic mice to produce mice with p53 inactivation in mammary tissue. Indeed, we observed p53 excision in the tissues of double transgenic mice. In addition, adenoviral vectors carrying cre recombinase are being used to inactivate p53. These approaches should provide a mouse mammary tumor model for studies of mammary tumor progression resulting from p53 mutation and for testing therapeutic interventions of mammary tumorigenesis.

Upon DNA damage, p53 protein becomes phosphorylated and stabilized, leading to subsequent activation of cell cycle checkpoints. It has been shown that ATM is required for IR induced phosphorylation on Ser15 residue of p53. Based on the involvement of p53 in mammary tumorigenesis and on the higher risk of ATM carriers for breast cancer, we have carried out studies to address the cancer susceptibility of ATM heterozygous and ATM null mammary epithelial cells by transplanting mammary gland to wild-type sibling mice. Initial studies have indicated differential checkpoint and apoptotic responses in cells harboring ATM mutation. These studies will establish whether ATM plays important roles in mammary tumorigenesis.

Lin, S-C. J., S. X. Skapek, and E. Y.-H. P.Lee Genes in the RB pathway and their knock in mice. Seminars in Cancer Biology 7:279-289, 1996.

Sean Post ***Mentor -- Dr. Eva Lee***

Recent studies indicate that breast cancer susceptibility genes,BRCA1 and BRCA2, are involved in DNA repair. Cells harboring mutations in either gene are hypersensitive to ionizing radiation (IR). Extensive genetic evidence in yeast indicates that DNA double-stranded breaks are processed by Rad50/Mre11 nuclease complex. It has also been shown that in response to IR, Rad50 assembles into nuclear foci. In mammalian cells, such IR-induced Rad50 foci are not observed in cells established from Nijmegen breakage syndrome (NBS). We and others have shown that the protein product of gene mutated in NBS, Nibrin, forms a stable complex with Rad50/Mre11 and the complex possesses nuclear activity. We demonstrated that IR-induced Rad50 redistribution requires ATM kinase activity. Rad50 is phosphorylated upon IR. Our preliminary studies indicate that such IR-induced Rad50 foci formation and phosphorylation are defective in A-T cells. In addition, IR-induced Rad50 foci formation is aberrant in some sporadiac cancers that express normal ATM, Rad50, Mre11, nibrin, BRCA1 and BRCA2 suggesting involvement of additional protein in this DNA damage response.

Mr. Post is a second year graduate student who is characterizing IR-induced Rad50 phosphorylation. How phosphorylation affects Rad50 function will be studied. In addition, cross-linking experiments will be carried out to investigate whether there is defective Rad50 protein complex formation in breast cancer cells. These studies will provide insights into the role of ATM

kinase cascade in the assembly of double-stranded breakage repair protein. Furthermore, characterization of components in the repair protein complex may lead to the identification of additional players involved in breast carcinoma.

Lei Zheng ***Mentor -- Dr.Wen-Hwa Lee***

In his work, Mr. Zheng explored the role of the retinoblastoma tumor suppressor (Rb) in the process of chromosome segregation. A yeast homologue (scHec1p) of the Rb-associated protein hHEC was shown to be essential for survival of yeast. The human HEC protein rescues the lethal phenotype of the null-mutation of sc*hec1* by complementing a critical role in modulation of chromosome segregation. A temperature-sensitive (ts) mutation of hs*HEC1* leads to a high frequency of errors in chromosome segregation. hsHec1p binds to Rb at an IxCxE motif specifically during M phase. In yeast carrying a ts allele of hs*hec1,* the expression of wild-type Rb reduced chromosome segregation errors by approximately 5-fold, suggesting that Rb enhances the fidelity of chromosome segregation. These results may aloso help explain why $Rb^{+/-}$ cells convert to $Rb^{-/-}$ at high frequency by loss of the wild-type *Rb* allele. How Rb and HEC facilitate chromosome segregation is the continuing pursuit of Mr. Zheng.

Zheng, L, Y Chen, DJ Riley, P-L Chen, and W-H Lee 1998 The retinoblastoma protein enhances the fidelity of chromosome segregation mediated by a novel coiled-coil protein hsHec1p. Submitted

Stephen Van Komen ***Mentor – Dr. Patrick Sung***

Mutations in the tumor suppressor genes *BRCA1* and *BRCA2* greatly increase the risk of breast cancers. Recent studies have indicated that the BRCA1 and BRCA2 proteins modulate the enzymatic machinery which repairs DNA double-strand breaks by homologous recombination. Ongoing studies address the mechanism of the recombinational repair machinery by dissecting the functions of various recombination factors from yeast and human cells. The trainee, Stephen Van Komen, has made highly significant progress toward achieving the goal of dissecting the functions of the human recombination factor Rad51B and the yeast recombination factor Rad54. Specifically, Mr. Van Komen has raised polyclonal antisera against the human Rad51B protein and, using baculoviral protein expression vectors, has expressed the Rad51B protein in insect cells and determined the kinetics of induction of Rad51B. Mr. Van Komen has recently developed a procedure for purifying the Rad51B protein to about 50% purity. In the coming months, Mr. Van Komen will refine the purification procedure, obtain highly purified Rad51B, and carry out its functional analysis. In addition, Mr. Van Komen has been making great stride toward characterizing the yeast recombinational repair factor Rad54 and its mutant variants. The results form Mr. Van Komen's studies will be important for delineating the role of recombinational DNA repair in breast cancer suppression.

John Leppard ***Mentor -- Alan Tomkinson***

Genes encoding DNA ligase III, its partner protein Xrcc1 and poly (ADP-ribose) polymerase(PARP) appear to be restricted to multicellular projects. Cell lines defcient in any of these proteins exhibit sensitivty to killing by alkylating agents and ionizing radiation, suggesting that they may function in the same pathway. This notion is suuprted by the detection of specific protein-protein interactions between DNA ligase III and Xrcc1 and between Xrcc1 and PARP. It is our working hypothesis that these proteins particpate in a pathway that detects and repairs genomic single-strand breaks. The goal of Mr. Leppard's project is to define the molecular mechanisms of this repair pathway. The initial approach will be to reconstitute the repair pathway with purified components .Mr. leppard has already contributed to a submitted

manuscript describing the DNA binding properties of DNA ligase III. He is currently developing expression systems that will allow us to purify the DNA ligase III-Xrcc1 complex.

This project is relevant to breast cancer since genomic instability is likely to be involved at many of the several stages of breast cancer progression leading to malignancy. Methods to intervene and stabilize the genome could prevent progression and spread of the disease. In addition, information about DNA repair processes in normal and cancer cells may lead to the development of treatment regimes that more effectively kill cancer cells and minimize damage to normal tissues and cells.

Publications:

Mackey, Z.B., Leppard, J., Menissier-deMurcia, Niedergang, C., Au, K., Chen, J. de Murcia, G. and Tomkinson, A.E. DNA ligase III is recruited to DNA strand breaks by a zinc finger motif homologous to that of poly (ADP-ribose) polymerase: Identification of two functionally distinct DNA binding regions within DNA ligase III (submitted, 1998)

6. Changes to the Program Faculty: The addition of Dr. Patrick Sung to the program faculty provides students with a unique opportunity to train in the laboratory of one of the world's experts in the biochemistry of DNA repair. His expertise in the reactions associated with recombinational repair of double-strand breaks in DNA is central to issues of genomic stability and cancer development. He is a recent recipient of a DOD Breast Cancer Research Idea Grant and Career Development Award both entitled "Interactions among BRCA1 and BRCA2 and components of the recomination machinery-I". He is an outstanding addition to the faculty of this training program.

7. Course Changes: None this year.

SUMMARY: The Breast Cancer Training Program continued to make excellent progress toward attracting and retaining excellently qualified students in breast cancer research. The students are receiving a high level of training in the modern research methods and theory. A total of 31 publications on breast cancer was achieved by students supported by the program. Combined with the basic instruction they receive in the Molecular Medicine Ph.D. Program, they will graduate as highly skilled researchers who will competitive effectively for post doctoral positions in the premiere breast cancer laboratories in the world.

OTHER SUPPORT

Funding Agency	Title & Grant Number	Project Period	Current Direct Costs
Chirgwin, J.M.			
VA	Associate Research Career Scientist	04/01/94-03/31/98	41,351
DOD	Role of Autocrine Motility Factor is Osteolytic Metastasis	04/01/98-03/31/01	63,391
NIH	Cell Biology of Plasma Transglutaminase Dr. John Chirgwin, Sponsor Dr. Manuel Santiago, P.I.	09/01/98-08/30/03	100,875
VA (pending)	Merit Award PTHrP and Prostate Cancer Metastasis to Bone	03/01/99-02/29/02	96,100
NIH (pending)	Regulation of Renin in Preeclamptic Hypertension	07/01/99-06/30/02	54,533
NIH (pending)	Endosomal Proteolytic Processing of Prorenin in Decidua	04/01/99-11/30/02	15,750
Fuqua, S.A.W.			
NIH/NCI	SPORE in Breast Cancer, Project 1, Clinical Tamoxifen Resistance: Mechanisms and New Agents 5P50CA58183-05	09/30/95-07/31/00	174,867
NIH/NCI	SPORE in Breast Cancer, Project 2, Heat Shock Proteins and Drug Resistance 5P50CA58183-05	09/30/95-07/31/00	141,695
NIH/NCI	Hypersensitive Estrogen Receptor in Premalignant Breast Disease R01CA72038-01	09/01/96-05/31/01	155,584
DOD	New Mechanisms of Tamoxifen Resistance in Breast Cancer Patients DAMD1794J4112	10/15/94-09/30/98	62,830
NIH/NCI	Markers of Breast Cancer Evolution and Progression, Program Project 2,	07/01/97-06/30/02	168,855

	Molecular Variants and Overexpression of ER in Clinical Breast Cancer Development 5P01CA30195-17		
NIH/NCI	Markers of Breast Cancer Evolution and Disease, Program Project 3, Development and Prognostic Factors in Premalignant Breast Disease 5P01CA30195-17	07/01/97-06/30/02	145,855
NIH/NCI	Training Program for Translational Breast Cancer T32CA70091-01	09/01/96-06/30/01	82,008
Komen Foundation (pending)	Predicting Response to Adjuvant Therapy with Tamoxifen	12/01/98-11/30/00	75,268
Lee, Eva			
NIH/NCI	Tumor Suppression Function of RB and p53 in Mammary Gland 5R01CA49649-10	05/10/89-04/30/99	155,428
NIH/NINDS	ATM Signaling and Neurodegeneration 1R01NS37381-01	05/01/98-02/28/01	153,963
The Council for Tobacco Research	Biological Function of the Retinoblastoma Gene in Small Cell Lung Carcinoma 2491AR2	08/08/97-12/31/98	30,435
Texas Higher Education Coordinating Board	ATM Protein in DNA Repair and in Breast Cancer Predisposition ATP3659-034	01/01/98-12/31/99	158,400
Komen Foundation	Study of ATM Expression in Breast Cancer Cells and Elucidation of ATM Function in p53-mediated DNA Damage Check Point Regulation, Postdoctoral Fellowship, Gopal Dasika	10/01/97-09/30/00	35,000
NIH/NINDS	ATM Network and ATM Function in Neuron,	03/01/97-02/28/99	63,100

	Postdoctoral Fellowship, Gang Chen 1F32NS10400-01		
NIH/SACI	Cancer Grant Antigen & Antibody Core, Antigen and Antibody Production Shared Resource 2P30CA54174-08	08/01/98-07/31/03	90,056
NIH/NCI (pending)	Program Project Grant, DNA Repair and Tumor Suppressor Genes	04/01/99-03/31/04	230,445
Lee, W.H.			
NEI	Molecular Basis of Retinoblastoma Formation 2R01EY05758-15	03/01/98-02/28/01	245,174
NCI	Cancer Suppression by the Retinoblastoma Gene 5R01CA58318-05	05/01/95-04/30/99	170,958
NCI	SPORE in Breast Cancer Project 5 – Tumor Suppressor Genes in Breast Cancer Development 5P50CA58183-06	08/01/95-07/31/00	143,924
NIH	Biomarkers of Breast Cancer—Project 5—BRCA-1 Malfunction in Breast Cancer	08/01/97-07/31/02	172,048
NIH (pending)	DNA Repair and Tumor Suppressor Genes (Eva Lee, P.I.), Project 5—BRCA1 and the DNA Repair Machinery	04/01/99-03/1/04	178,685
USAMRDC (pending)	Training Program in the Molecular Basis of Breast Cancer Research	08/01/98-07/31/03	163,629
Masters, B.S.S.			
NIH	Prostaglandin 19- and 20-Hydroxylation by Cytochrome P450 GM31296	07/01/97-06/30/01	148,550
NIH	Structural and Functional Modularity in Nitric Oxide Synthase GM52419	04/01/96-03/31/00	134,123
Welch Foundation	Structure-Function Relationships in the FAD-	06/01/96-05/31/99	52,000

	and FMN- Containing Enzymes, NADPH-Cytochrome P450 Reductase and Nitric Oxide Synthase AQ1192		
NIH	Structural Determinants of FAD- and FMN- Requiring Enzymes HL30050	04/01/98-03/31/02	156,300
Mundy, G.R.			
NIH	General Clinical Research Center-Program Director M01-RR01346	12/01/93-11/30/98	1,196,527
NIH	Training Program in Bone and Mineral Metabolism 32-AR07464	07/01/93-06/30/98	70,472
NIH	Cytokines and Bone Cell Function R01-AR28149	04/01/97-03/31/98	105,703
NIH	Effects of Tumors on the Skeleton, Project 3, Mechanisms of Bone Resorption and Hypercalcemia in Hematologic Malignancies 2-P01-CA40035	06/01/95-05/31/99	129,342
NIH	Effects of Tumors on the Skeleton, Administrative Core, Program Director 2-P01-CA40035	06/01/95-05/31/99	52,536
O'Connell, P.			
NCI/SACI	Molecular Biology Shared Resource 2P30CA54174	08/01/94-07/31/98	61,644
NIH	Susceptibility Genes in Mexican Americans R01DK47482	09/30/93-09/29/98	216,481
NCI	Translational Research in Breast Cancer – San Antonio 2P50CA58183	09/01/95-08/31/99	160,857
NIH	Genetic Epidemiology of NIDDM in Mexican Americans 2R01DK42273	04/01/96-03/31/01	418,134

NCI	Molecular and Genetic Epidemiology of Gliomas 2P01CA55261	01/01/96-12/31/01	81,433
NCI	Markers for Breast Cancer Evolution and Progression 4P01CA30195	07/01/97-06/30/02	101,955
NIH	CCR5 Regulation and Promoter Variants in HIV-1 Infection R01AI43279	04/01/98-03/31/03	178,837
NCI (pending renewal)	Molecular Biology Shared Resources 3P30CA54174	08/01/98-07/31/03	188,767
VA/DOD (pending)	Molecular Genetics of Prostate Cancer Progression	10/01/98-09/30/01	96,900
Osborne, C.K.			
NIH/NCI	SPORE in Breast Cancer 5P50CA58183	09/30/95-07/31/00	1,645,310
NIH/NCI	Markers of Breast Cancer Evolution and Progression 5P01CA30195-17	08/01/97-07/31/02	1,265,267
NIH/NCI	San Antonio Cancer Institute, Project Leaders 5P30CA54174-04	08/01/94-07/31/99	38,936
Komen	Mechanisms of Tamoxifen Resistance	10/01/96-09/30/99	35,000
NIH/NCI	Physician Scientist Training Grant in Oncology K12CA01723	09/01/97-08/31/02	359,845
Zeneca, Ltd.	A Double-blind Randomized Multicenter Trial Comparing the Efficacy and Tolerability of 125 and 250 MG of Faslodex in Post-Menopausal Women with Advanced Breast Cancer	11/01/96-04/31/99	67,000
NIH/NCI	Training Program in Academic Medical Oncology/Hematology 5T32CA9434	12/01/98-11/30/03	66,000
Sharp, Z.D.			
NIH	Nutritional Probe of the Aging Process, Co-	05/01/98-04/30/03	77,108

	Investigator 1P01AG14674-01A1		
American Institute for Cancer Research	The Efficacy of Diet and Chemopreventatives on Cancer Progression in a Novel Mouse Model Mimicking Human Tumorigenesis 98B067-REV	01/31/99-12/31/99	75,000
NIH (Pending)	Effect of Dietary Restriction on Gene Expression, Co-Investigator	02/01/99-01/31/04	123,200
NIH (Pending)	DNA Repair and Tumor Suppressor Genes (Eva Lee, P.I.); Project 5 Co-Investigator, BRCA1 and the DNA Repair Machinery	04/01/99-03/31/04	178,685
Sung, P.			
NIH/NIEHS	Yeast DNA Repair Genes and Proteins of the RAD52 Group 5R01ES07061-04	01/01/95-12/31/99	133,750
DOD	Breast Cancer Program, Interactions Among BRCA1, BRCA2, and Components of the Recombination Machinery	06/01/98-05/31/02	98,000
NIH (pending)	Program Project DNA Repair and Tumor Suppressor Genes (P.I.: Eva Lee); Project 1, Installation of DNA Double-Strand Break Repair.	04/01/99-03/31/04	178,046
Tomkinson, A.E.			
NIH	Cellular Function of Eukaryotic DNA Ligases 2R01GM047251-06	05/01/98-04/30/02	120,764
The Council for Tobacco Research	DNA Nucleotide Excision Repair in Eukaryotes 3786AR1	01/01/97-12/31/98	43,478
UNCF-Merck	Graduate Science Research Dissertation Fellowship Application; Fellowship	09/01/97-08/31/99	40,000

	for Zachery Mackey		
Nathan Shock	Cellular Functions of the Werner Syndrome Gene Product	07/01/98-12/31/99	15,000
San Antonio Cancer Institute	Characterization of the Protein-protein Interactions that are Required for the Function of DNA Ligase I in DNA Replication and DNA Excision	06/23/98-06/30/99	14,985
NIH (pending)	Proogram Project DNA Repair and Tumor Suppressor Genes (Eva Lee, P.I.); Project 2, Completion of DNA Repair	04/01/99-03/31/04	144,255